with which it was combined, the acidity attained in this way being equivalent to that of the soil water on the acid plots.

The authors attribute the continuance of the nitrification in these soils to the irregular distribution of the materials composing them; though acid as a whole, they still contain some calcium carbonate, each of the particles of which forms a centre for the nitrification process. The decline in fertility of the acid plots may be attributed to the repression of the normal bacterial activities of the soil and the encouragement of the growth of moulds.

The Origin and Destiny of Cholesterol in the Animal Organism. Part I.—*On the so-called Hippocoprosterol.*

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(From the Physiological Laboratory of the University of London.)

Introductory.

Since the discovery of cholesterol by Conradi in 1775, and its analysis by Chevreul in 1815, it has been found to be very widely distributed in the animal and in isomeric forms in the vegetable kingdom. It is found in small quantities in all protoplasmic structures, in blood, bile, sebum, and similar oily excretions of the skin, and is an especially abundant constituent of the white substance of brain and of the medullary sheath of nerve. But, although a considerable amount of work has been done, we have little or no definite knowledge of its physiological functions, and it is only in very recent times that a small glimmering of light has been thrown on its chemical constitution.

In 1862, Austin Flint* published a series of experiments by which he attempted to show that cholesterol is always more abundant in the blood coming from the brain than in the blood of the general arterial system, or in the venous blood from other parts; that its quantity is hardly appreciable in venous blood from the paralysed side in hemiplegia, and that it is

* "Experimental Researches into a new Excretory Function of the Liver," 'American Journal of Medical Sciences,' Philadelphia, 1862, new series, vol. 44, and "Recherches Expérimentales sur une Nouvelle Fonction du Foie," Paris, 1868.

separated from the blood by the liver. He also stated that in cases of serious structural disease of the liver, accompanied by symptoms pointing to blood poisoning, cholesterol accumulates in the blood, constituting a condition which he named cholesteræmia. From his experiments he came to the conclusion that cholesterol is a product of the metabolism of nervous tissues, that it is carried from the brain by means of the blood and excreted by the liver through the bile, and, finally, that "we know of no function which it has to perform in the economy, any more than urea or any other of the excrementitious principles of the urine." Flint's methods of analysis were, however, open to grave objection, and he draws sweeping conclusions from differences so slight that had his method of estimating cholesterol been capable of considerable accuracy, we should have hesitated to attribute much significance to the figures. Flint also observed* that the cholesterol of the bile undergoes a modification in the intestine, and is found in the fæces as "stercorine." Some support was lent to Flint's views by the experiments of Picot† in 1872 and Koloman Müller‡ in 1873. Picot reported a fatal case of "grave jaundice," in which he found a great increase in the proportion of cholesterol in the blood; and Müller injected into the veins of dogs 2·16 fluid ounces of a solution containing 69 grains of cholesterol, made by rubbing cholesterol with glycerine and mixing the mass with soap and water. In five experiments of this kind, he produced a complete representation of the phenomena of "grave jaundice."

How far Flint's views obtained general credence we do not know, for he is rarely quoted in physiological text-books, and we are unaware of any other extensive series of experiments on the subject; but B. Moore, in Schäfer's 'Text-book of Physiology' (1898), states that "according to Hoppe-Seyler cholesterol is a cleavage product, constantly formed in the metabolic changes in the living cell; and for this reason it is that cholesterol is invariably found as a chemical constituent of both animal and vegetable cells. Cholesterol does not easily undergo decomposition in the animal organism when once formed, and is principally excreted in the higher animals in the bile. It is formed in increasing quantity in tissue which is undergoing pathological change . . . . It is probably formed most in the metabolism of nerve tissue, taken up by the liver cells from the blood, and passed as an excretion into the bile ducts. Cholesterol is purely an excretion, and is not reabsorbed, but passes out of the body with the fæces." Some more recent observers

* *Ibid.*

† 'Journal de l'Anatomie,' Paris, 1872, vol. 8.

‡ "Ueber Cholesterämie," 'Archiv für Experimentelle Pathologie und Pharmakologie,' Leipzig, 1873, vol. 1, p. 213.

appear to hold the view that the cholesterol of the bile is formed either in the liver cells or in the cells lining the bile passages or both, but the evidence for this is not particularly convincing. That some cholesterol is formed somewhere within the liver, and not merely excreted by it, seems to be shown by an experiment of Jankau,* performed in Naunyn's laboratory. He injected cholesterol into dogs, and also gave it in their food, and ascertained that it had been absorbed; but he failed to find any increase of cholesterol in the liver tissue or in the bile. The analyses of the liver and the bile published by Kausch† from the same laboratory show no relationship between the amount of cholesterol in the gland and in its secretion. Thomas,‡ also working under Naunyn's direction, found that there is no relationship between the amount of cholesterol excreted and the kind of food taken. When, however, the dog under observation suffered from catarrh of the biliary passages, there was a marked increase in the cholesterol of the bile.

[Goodman,§ on the other hand, in an experiment on a dog with a permanent fistula, observed that the quantity of cholesterol found in the bile varied considerably with the nature of the diet, but that the amount of cholesterol in the food taken was without influence. Thus white of egg and calves' brain were equally efficacious in increasing the output of cholesterol, although the brain contains some 2 per cent. of its weight of cholesterol and the egg albumin practically none. He found, too, that intravenous injection of cholesterol did not increase the output in the bile, the result being in agreement with that of Jankau. Pribram‖ also has shown that in the case of rabbits which have been fed with cholesterol for some days and then killed, there is a decided rise in the cholesterol content of the blood, which, in consequence, exhibits an increased power of resistance to the hæmolytic effect of saponin.]¶

From these experiments, and from the fact that cholesterol is always found where cells are disintegrating, Naunyn strongly supports the view that cholesterol is produced, not in the liver cells, but from the cells of the passages,** and that it is a product of disintegration of their protoplasm. More recently, V. Harley and W. Barratt,†† in a series of experiments on the

* 'Cholelithiasis,' transl. by A. E. Garrod, New Syd. Soc., 1896.

† Dissertation, Strassburg, 1891.

‡ 'Cholelithiasis, *ibid.*

§ 'Hofmeister's Beiträge,' vol. 9, p. 91.

‖ 'Biochem. Zeit.,' 1906, vol. 1, p. 413.

¶ The passage within square brackets is added as the paper passes through the press.

** *Cf.* also Doyon and Dufort, 'C. R. Soc. Biologie,' 1896, vol. 10 (3), p. 487.

†† "An Experimental Enquiry into the Formation of Gall Stones," V. Harley and W. Barratt, 'Journ. Physiol.,' vol. 29, p. 341, 1903.

effect of introducing gall stones into the gall bladders of dogs, have shown that when the gall bladder is healthy the gall stones tend to disappear, while, on the other hand, when cholecystitis is present they remained unchanged.

In his Text-book of Physiology, Schäfer has also suggested that the constant presence of lecithin and cholesterin in the bile may well be associated with the destruction of the red corpuscles, which contain relatively considerable amounts of these substances.

It will be seen from the foregoing sketch that we know little or nothing definite as to the functions of cholesterol, and we certainly know nothing whatever of the breaking up of cholesterol in the animal body.

From the very general occurrence of cholesterol and its frequent association with lecithin, we cannot but think that it must play an important part in the cell economy, and must not be considered merely as a waste product. In this connection the experiments of Flexner, Noguchi,* Preston Kyes,† Abderhalden and Le Count,‡ on hæmolysis produced by cobra poison in the presence of lecithin and the inhibitory effect of cholesterin appear to us very significant.

Whatever may be the value of Flint's views on the cholesterol problem, he was correct in his statement that cholesterol is found in human fæces in the modified form of "stercorine."

This body was rediscovered in 1896, by Bondzynski§—who gave it the name of coprosterol—and was thoroughly investigated by Bondzynski and v. Humnicki.‖ It crystallises in long slender needles melting at 95° to 96° C. and behaves chemically as a saturated alcohol; it is dextrorotatory and gives colour reactions similar to those of cholesterol. Its formula, $C_{27}H_{48}O$, was confirmed by the analyses of a large number of derivatives, and its discoverers regarded it as a dihydrocholesterol formed by bacterial reduction in the intestine. They fed a man with cholesterol and found that it was excreted mainly as coprosterol, and, later, Müller¶ proved that on a milk diet, in which

* "Snake Venom in Relation to Hæmolysis, Bacteriolysis, and Toxicity," 'Journ. of Experim. Med.,' vol. 6, No. 3, 1902.

† "Ueber die Wirkungsweise des Kobragiftes," 'Beit. klin. Wochenschr.,' Nos. 38 and 39, 1902; also "Lecithin und Schlangengift," 'Zeit. f. physiol. Chemie,' vol. 41, p. 273, 1904; also "Zur Kentniss der Kobragift aktivierenden Substancen," 'Berl. klin. Wochenschr.,' Nos. 2—4, 1903.

‡ "Die Beziehungen zwischen Cholesterin, Lecithin, Kobragift, Tetanuslaxin, Saponin und Solanin," 'Zeitschr. f. experim. Path. u. Therap.,' vol. 2, p. 199, 1905.

§ "Cholesterol of Human Fæces," 'Ber. der Deut. Chem. Ges.,' 1896, vol. 29, p. 476.

‖ "The Fate of Cholesterol in the Animal Organism," 'Zeit. Physiol. Chem.,' 1896, vol. 22, p. 396.

¶ "Reduction of Cholesterol to Coprosterol in the Human Intestine," 'Zeit. physiol. Chem.,' 1900, vol. 29, pp. 129—135.

putrefactive changes in the intestine are reduced to a minimum, the cholesterol of the body is excreted unchanged. The reduction of cholesterol in the intestine thus seems established in the case of man, and Bondzynski and Humnicki, continuing, examined the fæces of the dog and the horse.* In the case of the dog, they stated that the cholesterol of the bile was excreted unchanged, but in that of the horse intestinal reduction went much further than in man, and a cholesterol-like body, $C_{27}H_{54\text{ or }56}O$, was found, crystallising in microscopic needles melting at 74° to 75° C.

This hippocoprosterol, as it was called, was further examined in 1905 by Wilenko.† The results of his work go to prove that the excrement of the horse contains two isomeric bodies, $C_{27}H_{52\text{ or }54}O$, one readily soluble, the other much less soluble, in 97 per cent. alcohol. These he designated as α and β-hippocoprosterol respectively. The α body crystallises in minute rhombic tables similar to cholesterol crystals. When dry it forms silky scales which are as soft as wax and melt at 66° to 67° C. The β isomer appears to be identical with Bondzynski and Humnicki's hippocoprosterol, though Wilenko gives its melting point as 56° instead of 74°.

In order to throw some further light on the origin and functions of cholesterol in the animal economy, it appeared to us in the first instance essential, more especially considering the discrepancies and scantiness of the work of previous observers, to make a comparative study of the forms in which cholesterol is found in the fæces of different animals and to determine to what extent the substances thus excreted are dependent upon the food taken. In the present paper we give an account of our experiments on the fæces of herbivorous animals, those examined being the horse, cow, sheep, and rabbit.

Method of Experiment.

The material was obtained from grass-fed animals (Hampshire) and was sometimes dried directly in the water oven, but generally spread out in thin layers and allowed to dry in the air. In order to deal effectively with such a light, bulky substance, we employed large metal extractors capable of holding 2 to 5 kilogrammes of material. They were made on the Soxhlet pattern, fitted with long metal condensers, and the ether vessel was enclosed in an air chamber warmed with incandescent electric lamps. The dried material was extracted usually for five to six days with ether, the dark green solution obtained diluted, if necessary, with more ether and at once saponified with sodium ethylate in alcoholic solution according to the method of Kossel

* *Ibid.*

† "Hippocoprosterol," M. Gittelmacher Wilenko, 'Bull. International de l'Académie des Sciences de Cracovie,' No. 1, Jan., 1906, p. 20.

and Obermuller: care had, however, to be taken not to allow the alcohol added to amount to more than 1/10 to 1/12 of the volume of the ether, lest any of the hippocoprosterin should be precipitated along with the soaps. The mixture was well shaken, allowed to stand overnight, and the soaps filtered off and washed with ether. The filtrate was then shaken, first with an equal bulk of water to remove alkali and alcohol, and then with water containing potash to remove the last traces of soap. The ether solution was finally separated off, dried, and evaporated.

The crude extract was dark red, liquid at 100° C., and had a pungent smell like that of wood spirit. It dissolved in boiling alcohol (with the exception of a small residue soluble in benzene), giving a deep red solution which could be partially decolorised by treatment with charcoal. The charcoal, however, subsequently required repeated extraction with alcohol, as it obstinately retained the substance. The pale yellow filtrate, on cooling, set to a bulky gelatinous mass which was difficult to filter and left a solid which, on drying, shrank up to light flakes of impure hippocoprosterol. This was dissolved in ether and precipitated by alcohol, the process repeated, if necessary, and the nearly white material dissolved in ether and allowed to crystallise by slow evaporation.

The alcoholic mother liquors obtained in the above processes were subjected to further crystallisation till only oily matter remained.

Examination of Horse Excrement.

The hippocoprosterol obtained, as above, was purified by crystallisation from benzene or ethyl acetate, and finally from ether. Sometimes it was necessary to use charcoal again before the last traces of colour could be removed, but often this could be dispensed with. The yield was 0·2 per cent. of the dry fæces.

Hippocoprosterol is a light, white substance, which may be powdered, but cakes together under slight pressure. It dissolves at the boiling temperature in all the usual solvents (except water), but comes out again almost completely in the cold. Thus its solubility in benzene at 16° C. is only 0·32 part per 100 parts of solvent. From ethyl and methyl alcohol, ethyl acetate, glacial acetic acid and acetic anhydride, it comes out as a white jelly; from ether, petrol ether, benzene and chloroform in a more powdery form. From strong solutions it crystallises in microscopic needles grouped in stars, rosettes, and comet-shaped clusters, but on slow evaporation these clusters may be obtained 1 to 2 mm. in diameter. If a dilute ethereal solution be allowed to evaporate spontaneously, large transparent masses of crystals are obtained, consisting of fan-shaped groups of needles springing from a common base. It melts

at 78°·5 to 79°·5 C., and solidifies at 77°, a characteristic property. It is optically inactive, and does not give the cholesterol colour reactions. These observations are at variance with the statements of Bondzynski and Humnicki, who attribute to hippocoprosterol a slight dextrorotation and a green colour reaction in a modified Salkowski test, but seeing that their product melted at 74° to 75° C., it probably contained some impurity which produced these effects. Wilenko's β-hippocoprosterol, which is undoubtedly identical with ours, he describes as melting at 56°, a discrepancy we are unable to understand, since on elaborate purification of our product we failed to obtain any alteration in the melting point. Hippocoprosterol does not absorb bromine in carbon bisulphide solution. Dry bromine in a sealed tube at 100° C. is without action, but at 170° C. various substitution products are obtained.

For analysis the substance was dried *in vacuo* at the ordinary temperature, and was burnt in a copper boat filled with coarse copper oxide.

I. 0·1936 gave 0·5823 CO_2 and 0·2473 H_2O.
II. 0·1447 „ 0·4352 CO_2 „ 0·1738 H_2O.

	Found.		Calculated for	
	I.	II.	$C_{27}H_{54}O$.	$C_{27}H_{56}O$.
C	82·03	82·03	82·14	81·73
H	14·13	14·11	13·80	14·24

A molecular weight determination, carried out by the cryoscopic method, showed that it possesses the simple formula :—

0·8321 gramme in 13·36 grammes of naphthalene (constant = 70) gave a depression of 1°·157.

Molecular weight found, 377. $C_{27}H_{54}O$ requires 396.

Hippocoprosterol Acetate.—One part of hippocoprosterol was mixed with two parts of fused sodium acetate and five to six parts of acetic anhydride, and heated to boiling for half an hour. The product was poured into water, and the precipitated acetate dissolved in acetic ether. It came out from this in flocks, which consisted of microscopic needles resembling the original substance. The yield was almost quantitative.

The acetate is moderately soluble in alcohol and acetic ether, easily in ether, benzene and petrol. From the latter it was obtained in shining flakes, which were sticky, caking together under the least pressure. Melting point 61° to 62° C.

On analysis, the following figures were obtained :—

I. 0·1719 gave 0·5035 CO_2 and 0·2061 H_2O.
II. 0·1831 „ 0·5333 CO_2 „ 0·2181 H_2O.

	Found.		Calculated for	
	I.	II.	$C_{27}H_{53}O.CO.CH_3$.	$C_{27}H_{35}O.CO.CH_3$.
C	79·88	79·43	79·73	79·37
H	13·32	13·24	12·93	13·33

On saponification of the acetate with sodium ethylate, hippocoprosterol was obtained, melting at 79° C.

Hippocoprosterol Benzoate.—The hippocoprosterol was mixed with an equal weight of benzoic anhydride, and heated to 160° for two hours in an open vessel. The product was boiled with alcohol, and on cooling the benzoate separated in thick clots. These were recrystallised several times from petrol, and finally from ethyl acetate. Hippocoprosterol benzoate crystallises in microscopic needles, which in mass appear as sticky lumps. It is difficultly soluble in alcohol and ethyl acetate (but more so than the mother substance), easily in ether, petrol, and benzene. The melting point is 58°·5 to 59°·5 C. In concentrated ether solution it proved optically inactive, and on analysis gave the following figures:—

0·1799 gave 0·5395 CO_2 and 0·1975 H_2O.

	Found.	Calculated for	
		$C_{27}H_{53}O.CO.C_6H_5$.	$C_{27}H_{55}O.CO.C_6H_5$.
C	81·79	81·85	81·52
H	12·20	11·73	12·09

Two grammes of the benzoate were saponified with sodium ethylate and yielded hippocoprosterol, melting at 79°.

*Hippocoprosterol Cinnamate.**—Five parts of hippocoprosterol were mixed with three parts of cinnamyl chloride and heated to 140° for one hour. The product was boiled out with alcohol, and the solution on cooling deposited the cinnamate in masses of minute needle-shaped crystals which, when dry, appeared sticky, caking together under pressure. It is very soluble in benzene, moderately in acetic ether and petrol, and difficultly in alcohol. The melting point is 62° C. On analysis the following figures were obtained:—

0·2040 gave 0·6143 CO_2 and 0·2132 H_2O.

	Found.	Calculated for	
		$C_{27}H_{53}O.CO.C_8H_7$.	$C_{27}H_{55}O.CO.C_8H_7$.
C	82·17	82·37	82·05
H	11·61	11·53	11·81

* This paragraph is added as the paper passes through the press.

Hippocoprosterol behaves, therefore, as a saturated alcohol. The hydroxyl group, however, is not readily replaceable by chlorine. When ground up with phosphorus pentachloride in the cold, no action takes place, though on adding petrol and boiling some hydrochloric acid is evolved, but we have not succeeded in preparing the chloride, either by this method or by the use of thionyl chloride. Various substances appear to be formed, which we are at present investigating.

Examination of the Alcoholic Mother Liquors.

The residues remaining after separation of the hippocoprosterol were subjected to a careful examination, first with the object of isolating the α-hippocoprosterol (m.p. 66° to 67°) described by Wilenko, and secondly to discover whether any of the cholesterol of the bile was present.

As an example, 2 kilogrammes of dry dung obtained in the summer yielded 3·65 grammes or 0·18 per cent. of hippocoprosterol, and 8·1 or 0·4 per cent. of a dark red buttery residue. The latter was dissolved in alcohol and allowed to evaporate. The first crops of material obtained gave melting points varying between 65° and 70°, but small quantities of pure hippocoprosterol could always be obtained from these. The second crop (0·2 gramme) gave a body which dissolved easily in all solvents, except dilute alcohol, from which it crystallised readily in microscopic hexagonal plates. It melted at 136° to 137°, absorbed bromine in carbon bisulphide solution, and gave the Salkowski and Liebermann colour tests. It thus agreed closely with the sitosterol of Burian.* The acetate of the body, however, did not confirm this. It was made in the usual way with acetic anhydride and sodium acetate, and came out of dilute alcohol in brilliant glistening leaves consisting of six-sided plates. In all its properties, however, it agreed with the original substance, except that it was less soluble in absolute alcohol. It gave a constant melting point of 136°, which could not be altered by repeated crystallisation. Sitosterol acetate melts at 127°·5. The third crop (0·1 gramme) was white, and after several crystallisations from 80-per-cent. alcohol appeared under the microscope as long thin plates like sword blades. These melted sharply at 161° to 162° and gave Liebermann's colour test. To identify it if possible with the caulosterine obtained by Schulze and Barbieri† from the shoots of the yellow lupin (m.p. 158°), it was heated with benzoic anhydride, but no crystalline benzoate could be obtained. The concentrated alcoholic filtrate still showed traces of solid matter, but this could not be

* From germinating wheat, 'Monatshefte f. Chemie,' 1897, vol. 18, p. 551.

† 'Journ. Prak. Chem.' (2), vol. 25, p. 159.

obtained free from oil. The alcohol was accordingly evaporated off, and the buttery residue, which dissolved very easily in most solvents, was treated with 75-per-cent. alcohol, in which it was more difficultly soluble. From this a white solid body was obtained in small quantity (less than 1 per cent.), which gave Liebermann's test and crystallised from dilute alcohol in the sword blade plates described above. From ether and petrol it dried up to rosettes of needles which softened at 145° and melted clear at 154° to 155°. This melting point could not be raised by repeated crystallisation. To discover whether it was identical with Tanret's ergosterol,* it was converted into the acetate by heating with acetic anhydride and sodium acetate. The product, which was very soluble in petrol, crystallised from dilute alcohol in microscopic rectangular plates. It, however, melted at 78° to 80° C., whereas Tanret's acetate melted at 169° to 176°.

After separation of the solid bodies as above, the alcoholic solution leaves, on evaporation, a very dark red thick oil, which, in the case of the horse, has not been further investigated. It is important, however, to emphasise that, although we have extracted some 15 kilogrammes of dried fæces, we have not been able to obtain either the α-hippocoprosterol of Wilenko, or to detect any trace of cholesterol, microscopically or otherwise. Instead, we have obtained in minute quantity high melting bodies, which, so far as they could be examined, appeared to belong to the phytosterol, or vegetable cholesterol, group.

Examination of the Excrement of the Cow and Sheep.

The material was obtained from grass-fed animals, and was extracted and worked up as before. The chief product was in each case a body identical in all its properties with the hippocoprosterol described above. The yield amounted to 0·15 per cent. of the dry dung in the case of the cow, and 0·3 per cent. in that of the sheep. The identity was proved by the crystalline form, solubility, melting points, mixed melting points, and analysis.

I. Cow ..... 0·2383 gave 0·7168 CO_2 and 0·3025 H_2O.
II. Sheep ..... 0·1578 „ 0·4780 CO_2 „ 0·2028 H_2O.

	Found.		Calculated for
	I.	II.	$C_{27}H_{54}O$.
C	82·02	82·61	82·14
H	14·10	14·28	13·80

The cow also gave about 0·3 per cent. of a dark red oil similar to that of

* The ergosterol described by Tanret ('Annales de Chim. et de Physique,' series 6, vol. 20, p. 289) crystallises from alcohol in plates, from ether in needles. M. p. 154°.

the horse, while the sheep gave 0·4 per cent. of a yellow vaseline-like substance smelling strongly of hay.

Examination of the Excrement of the Rabbit.

The material was obtained in the summer from wild rabbits. Treated as before, from 3 kilogrammes of the dried fæces, 6·2 grammes, or 0·21 per cent., of pure hippocoprosterol were obtained. On analysis it gave the following results :—

0·1656 gave 0·4974 CO_2 and 0·2135 H_2O.

	Found.	Calculated for $C_{27}H_{24}O$.
C	81·91	82·14
H	14·33	13·80

The oils left after complete separation of the solid matter weighed 23·26 grammes, or 0·77 per cent.

Mr. G. W. Ellis, at our suggestion, attempted to ascertain the composition of these residues by a fractional distillation *in vacuo*, but the process proved tedious and difficult and led to no very definite results. At first the liquid simply frothed over, but on returning the distillate and repeating several times the frothing became less marked, and under a pressure of less than 1 mm. it distilled over between 98° and the temperature at which the glass softened without the slightest charring. After elaborate and repeated fractionation, four main fractions were obtained, boiling around the following temperatures:—(1) 98°, (2) 164° to 168°, (3) 215° to 220°, (4) 260° to 265°. In the flask there remained a transparent, yellow, brittle, resinous substance which was not decomposed at the softening point of glass.

Fraction 1 consisted of about 2 c.c. of a pale yellow, fairly mobile oil, with a smell recalling that of pine oil.* On combustion it was found to contain 82·13 per cent. carbon and 11·96 per cent. hydrogen.

Fraction 2 consisted of about 3 c.c. which, on long standing, deposited a trace of crystalline matter. It had a very faint turpentine odour and reduced ammoniacal silver solution in the cold, markedly on heating.

Fraction 3 consisted of about 5 c.c. of a very thick oil, smelling faintly of hay, and only just mobile at the ordinary temperature.

Fraction 4 was the largest and consisted of a pale yellow, sticky, solid (at the ordinary temperature), which showed no signs of crystallisation after many months' standing. On combustion it gave the following figures, which agree closely with those required for cholesterol :—

* The rabbits from which these fæces were obtained lived on the border of a pine wood.

I. 0·2449 gave 0·7562 CO_2 and 0·2547 H_2O.
II. 0·2694 „ 0·8343 CO_2 „ 0·2908 H_2O.

	Found.		Calculated for
	I.	II.	$C_{27}H_{44}O$.
C	84·21	84·05	84·37
H	11·56	11·99	11·46

All attempts to prepare a crystalline acetate or benzoate of this substance failed.

In the course of the investigation we noticed that samples of fæces collected during the winter season, when the animals were not fed entirely on grass, gave a lower yield of hippocoprosterol. Furthermore, an examination of the fæces of domestic rabbits, fed on cabbage, made for us by Mr. G. D. Knox, showed that with this food a different product was obtained, an account of which we reserve for a future communication. We therefore were led to suspect that the hippocoprosterol might be a constituent of the grass on which the animals fed.

Examination of Grass.

3·2 kilogrammes of the blades of grass obtained from the cuttings of a well-kept cricket pitch, fairly free from clover and other plants, were dried in thin layers in the air and extracted as before. The extract was dissolved in alcohol and decolorised with charcoal. The solution was now pale yellow and deposited a gelatinous solid mass which was readily worked up by methods previously described and obtained pure. The yield was 8 grammes, or 0·27 per cent., of the dry material. This body proved identical with hippocoprosterol, an important point which was confirmed by the following experiments:—

(*a*) six grammes of the body were crystallised from benzene into three fractions, *each* of which gave a melting and solidifying point (79° and 77° respectively), the same as that of hippocoprosterol.

(*b*) Equal weights of the body from grass were mixed separately with equal weights of that from the horse, cow, sheep, and rabbit. The melting points remained unchanged. Finally, a sample from each of the five sources was mixed and the same result obtained.

(*c*) The acetate and benzoate were made as before and proved identical with those from hippocoprosterol. They were saponified and yielded hippocoprosterol, melting at 79°.

(*d*) On analysis, the following figures were obtained:—

I. 0·1924 gave 0·5758 CO_2 and 0·2464 H_2O.
II. 0·1742 „ 0·5234 CO_2 „ 0·2230 H_2O.

	Found		Calculated for $C_{27}H_{54}O$.
	I.	II.	
C	81·62	81·94	82·14
H	14·23	14·22	13·80

The mother liquors from this product yielded a considerable quantity of reddish oily matter, similar to that found in the fæces.

It would, therefore, appear that hippocoprosterol is not an animal product, but is a constituent of the grass food which is passed unchanged. In order to confirm this conclusion, and also to ascertain whether any cholesterol or derivative of it which we might have missed in our previous experiments was excreted by the animal, we made a series of experiments in which a domestic rabbit was fed on grass which had been thoroughly extracted with ether.

Experiment 1.—A rabbit, weighing 2·1 kilogrammes, was fed with 315 grammes of extracted grass, slightly moistened with water, during 14 days. A very small quantity of bran was given in addition. The animal took the grass readily, and at the end of the experiment had only lost 0·1 kilogramme in weight, and appeared to be in good health. The weight of dry fæces obtained was 128 grammes. This was extracted in the usual way, and the extract was found to have a somewhat fœtid odour not noticed with ordinary dung. One gramme of unsaponifiable matter was obtained, the greater portion of which was soluble in alcohol. On standing, a very small quantity of red crystalline matter was deposited, but the bulk of the substance eventually separated as a non-crystalline red oil. No trace of hippocoprosterol was discovered, and the small quantity of crystalline matter referred to after purification was obtained from dilute alcohol in the form of glancing white leaf-like crystals and from ethyl acetate as needles, which melted rather indefinitely at about 129°. Under the microscope the crystals from alcohol showed the form of hexagonal plates, recalling those of phytosterol, but the quantity was too small for further investigation.*

Experiment 2.—A rabbit, weighing 2·1 kilogrammes, was fed during 18 days on extracted grass with a little bran. The quantity consumed weighed before extraction 2·8 kilogrammes, and was of a somewhat coarser

* This substance was derived from the bran given to the animals, as on extraction of a sample of bran we obtained a body, crystallising in the same forms, which melted at 137°·5 C., and appeared to be identical with Burian's sitosterol.

description than that previously used; 982 grammes of dried fæces were obtained, and were treated as before. The residue obtained weighed 5·64 grammes. From this we isolated 0·25 gramme of hippocoprosterol, and the mother liquors on evaporation deposited an oil, which on standing showed under the microscope traces of crystalline matter in the form of six-sided plates and clusters of sword blades, but in too small quantity for further examination and identification.*

General Conclusions.

1. Hippocoprosterol isolated from the fæces of the horse by Bondzynski and others is not a product of animal metabolism, but is a constituent of the grass taken as food, and is passed unchanged by all herbivorous animals when fed on grass. The name is, therefore, misleading, and we propose to rename the substance chortosterol (χόρτος, grass).

2. Chortosterol is an alcohol having the formula $C_{27}H_{54}O$ or $C_{27}H_{56}O$. It is not possible at present to decide definitely between these, though our analyses in every case agree better with the former.

3. If we consider the numerous vegetable cholesterols which have the properties of unsaturated monatomic alcohols and the formula $C_{27}H_{44}O + H_2O$ as isomeric substances constituting the phytosterol group, chortosterol cannot be regarded as a simple reduction product of any one of them in the same way that coprosterol is supposed to be related to cholesterol. It is possible that the substance may be derived from some member of this group, or *vice versâ*, by some rearrangement of the ring structure during the development of the plant. We are at present engaged in some experiments on this point. This is, perhaps, supported by the fact that chortosterol, unlike other members of the cholesterol or phytosterol groups, gives none of the usual colour reactions, for Windaus† has shown that when the unsaturated open side chain of the cholesterol and phytosterol molecules is condensed to a ring, the products obtained show the colours feebly‡ or not at all.

4. In all the experiments we have made we have never found any cholesterol in the fæces of the herbivora. If the view of Flint and other observers that cholesterol is an excrementitious product got rid of in the fæces through the agency of the bile, we should certainly have expected

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† 'Ber.,' vol. 40, pp. 2637 and 3681 (1907).

‡ *Cf.* Diels and Abderhalden, 'Ber.,' vol. 39, p. 884 (1906).

in the very large quantities of material examined to have obtained considerable quantities of cholesterol as such, or in a modified form as in the human subject. In the cow, for instance, every 100 c.c. of bile contains approximately 0·07 gramme of cholesterol, and supposing in this animal only $2\frac{1}{2}$ litres are poured into the intestine per day, this would mean a daily excretion of nearly 2 grammes, which we could not possibly have failed to discover. It follows, therefore, that cholesterol of the bile must either have been reabsorbed with the bile salts in the gut, or else destroyed. We are at present carrying out experiments on this subject with herbivora and other animals, an account of some of which we hope shortly to have the honour of laying before the Society.

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